

## Written Reply

To: NANAJO Satomi,

Examiner of the Patent Office

## 1. Designation of the International Application

5 PCT/JP2004/005071

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## 20 5. Contents of Reply

The Examiner has recognized claims 1 to 6 in the present application as neither being novel nor involving any inventive step for the following three reasons.

(1) Document 1 discloses a method for obtaining human  
25 chondrocyte mass by multilayer culturing chondrocytes

isolated from a cartilage piece and a cartilage therapy material. Although it is not clearly indicated in document 1 that chondrocytes are co-cultured together with perichondrium, it is recognized that a cartilage tissue is generally coated with perichondrium and thus a cartilage piece collected by a commonly employed method essentially has perichondrium bonded thereto. Thus, it is considered that chondrocytes are cultured together with perichondrium in the method of document 1 too. Such being the case, the inventions as claimed in claims 1 to 6 are not novel.

(2) Documents 2 and 3 disclose methods of monolayer culturing chondrocytes isolated from human cartilage pieces. Since it is recognized that these human cartilage pieces also had perichondrium bonded thereto, claims 1 to 4 are not novel for the same reason as discussed in (1).

(3) Document 1 discloses that cells are multilayer cultured to give a cell mass and the cell mass is embedded in collagen or the like. Accordingly, it is self-evident to multilayer culture chondrocytes, which have been monolayer cultured as reported in documents 2 and 3, to give a cell mass and embed the cell mass in collagen or the like to give a therapy material. Thus, claims 5 and 6 involve no inventive step.

However, the applicant cannot accept the Examiner's recognition as discussed above. Now, the applicant's opinions will be offered.

5 To the reason (1):

1) Concerning "isolation of chondrocytes", it is stated in document 1 (page 9, lines 1 to 7):

"(1) The sampled cartilage tissue is minced;

(2) kept stationary almost overnight in a medium containing  
10 trypsin at 4°C;

(3) incubated with type II collagenase for 1 to 6 hours at  
37°C;

(4) agitated in a BSA-containing medium for several hours,  
and filtered with a 100 µm filter;

15 (5) to give isolated chondrocytes." (document 1, page 4)

By the treatments (2) to (4), the chondrocytes are separated from the perichondrium and the extracellular tissue, with which they have coexisted, and fractionated by the filtration.

2) In the invention of the present case, on the other  
20 hand, it is stated:

"1) a cartilage tissue is excised and diced;

2) allowed to stand in a medium containing type II collagenase  
at about 4°C overnight and then shaken at 37°C for 4 hours;

3) thus treated tissue is centrifuged and the obtained  
25 precipitate is employed in the culture." (description of

the present case, page 7)

Compared with the treatment method of document 1, the method of the present case differs in "performing no trypsin treatment" and "performing no filtration with a filter". In the method according to the invention of the present case, namely, digestion with "trypsin" is not performed to allow the coexistence of chondrocytes with perichondrium and "centrifugation" is employed as a substitute for "filtration with a filter" to give chondrocytes coexisting with perichondrium pieces.

3) Thus, it cannot be concluded "chondrocytes are cultured together with perichondrium in the method of document 1 too".

To the reason (2):

1) Document 2 reports a method of isolating and culturing an articular cartilage, in particular knee articular cartilage. However, it is described therein (page 258, lines 1 to 3) "an articular cartilage consists of chondrocytes and extracellular matrix made of type II collagen, proteoglycans, etc. located around them". That is to say, an articular cartilage has no perichondrium.

The fact "an articular cartilage has no perichondrium" is also clearly mentioned in, for example, *Hyojun Seikeigekagaku*, 3rd ed., page 27 (Igaku Shoin, published on

1998.10.15) (APPENDIX 1).

Thus, it cannot be concluded "chondrocytes are cultured together with perichondrium" in the method of document 2 too.

2) Document 3 is entitled "Treatment of Deep Cartilage  
5 Defects in the Knee with Autologous Chondrocyte Transplantation". Further, it presents "collection and cultivation of knee articular cartilage" (page 890, FIG. 1) and states "cartilage slices were obtained through an arthroscope from - - - the upper - - - of the damaged knee"  
10 (the same page, left column, lines 8 to 12 from the bottom) followed by the illustration of the cultivation thereof. Therefore, document 3 reports the cultivation of articular cartilage, in particular, knee articular chondrocytes too.

As described above, knee articular cartilage has no  
15 perichondrium and, therefore, it cannot be concluded "chondrocytes are cultured together with perichondrium" in the method of document 3 too.

Although "articular cartilage" is cited as an example  
20 of "human cartilage tissue having perichondrium bonded thereto" in the description of the present case (page 6, line 20), this is a mistake that is to be deleted and corrected in the written amendment filed separately.

25 To the reason (3):

Although the Examiner points out "Document 1 discloses that the cells are multilayer cultured to give a cell mass and the cell mass is embedded in collagen or the like for fixation.", the cell mass obtained by the culture in document 1 is a mixture of "human chondrocytes" with "feeder cells, for example, chondrogenic-stage perichondral cells from a mammalian fetus, especially preferable are chondrogenic-stage perichondral cells from a 13-day-old murine fetus" (document 1, page 2, lines 18 to 26, in particular, lines 24 to 27). In contrast, the cell mass obtained by the culture method of the present invention is a mixture of "human chondrocytes with perichondrium (cells)" containing no hetero animal cells. Namely, it is a "cell mass" free from any risk of rejection or unexpected contamination with a virus, etc. That is, the cell mass obtained by the culture method of the present invention obviously differs from the cell mass described in document 1. As a result, "a therapy material comprising the cells, which are obtained by the culture method according to the present invention, embedded in collagen or the like" exerts a technical merit "being free from any risk of rejection or unexpected contamination with a virus, etc." and obviously differs from the "therapy material" reported by document 1.

As discussed above, the applicant asserts that the present application should be re-examined.

6. List of Attached Document

APPENDIX A: *Hyojun Seikeigekagaku*, 3rd ed., page 27, Table of  
Contents and Publication Data (Igaku Shoin  
published on 1998.10.15)

5

STANDARD ORTHOPAEDICS

## 標準整形外科学

*Hyojun Seikeigekagaku,*

〔編 集〕

信州大学教授 元千葉大学教授 神戸大学教授  
寺 山 和 雄 井 上 駿 一 広 畑 和 志

〔執 筆〕

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(執筆順)

第 3 版

3rd ed.

医学書院

Igaku Shoin

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## III. 関節の構造と生化学 27

図 1-20 関節の模式図

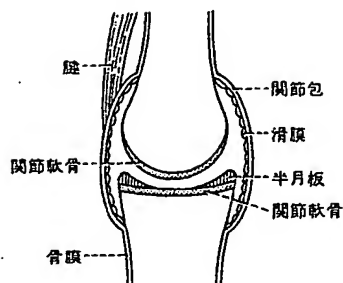


図 1-21 関節腔の形成

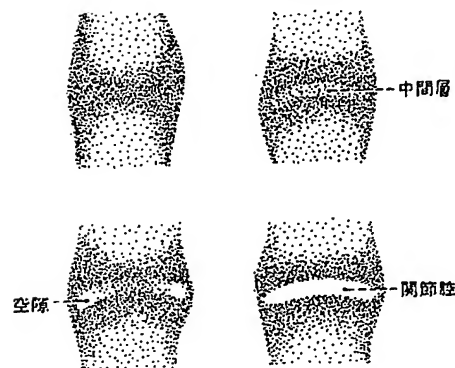
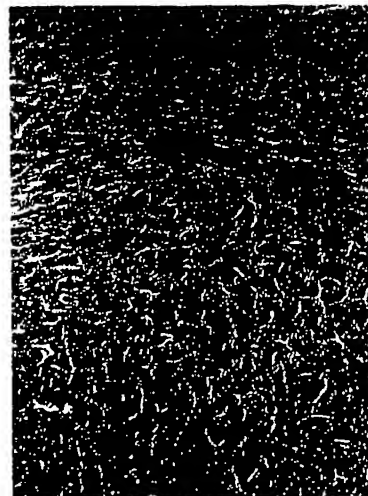


図 1-22 関節軟骨の走査電顕像 (×400)



周囲の靱帯も関節腔周囲の靱帯から分化したものである。

## 2. 関節軟骨

### 2. Articular Cartilage

#### a. 関節軟骨の構造

##### a. Structure of Articular Cartilage

滑膜関節は一般に硝子軟骨 hyaline cartilage からできており、その厚さは個体の体重に相関するといわれる。ヒトの膝関節や股関節では 2~4 mm である。成熟した関節軟骨は神経、血管、リンパ管を欠き、滑液によって栄養される。

#### 1) 関節表面の構造 articular surface

→ 骨膜、軟骨膜、その他の膜様構造をみない。肉眼的には関節表面はきわめて平滑であるが、走査電子顕微鏡で観察すると非常に凹凸不整である(図 1-22)。すなわち関節の表面には高さ 0.4~0.5 mm のうねり (undulation) があって、さらにそのうねりには 20~30 μ の深さの凹み (pit, depression) が多数みられる。このものは軟骨細胞窩に一致すると考えられている。これらの凹みは潤滑を説明するのに好都合である。

#### 2) 軟骨細胞

関節軟骨における軟骨細胞の密度はきわめて低い。成熟した関節軟骨は軟骨細胞の形態、配列や基質の状態から、次の 4 層に分けられる(図 1-23)。

① tangential (gliding) zone: 最表層で扁平な線維芽細胞様の軟骨細胞が関節表面に平行にならび、基質はプロテオグリカン多糖にきわめて乏しい。

② transitional (intermediate) zone: やや楕円形の軟骨細胞が不規則に配列し、プロテオグリカン多糖を組織化学的に基質に証明する。

③ radial zone: 円形の軟骨細胞が関節表面に

Membrane like structure, such as periosteum, perichondrium and so on. is not found.

## 標準整形外科学

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